

Appendix A
Claims After Entry of Foregoing Amendment

1. A nucleic acid sequence for enhancing expression of a useful gene incorporated into a gene expression vector for enhancing expression of a useful gene comprising a nucleic acid sequence corresponding to a 5'-untranslated region of a viral gene or a fragment or a variant thereof.

2. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises at least one pyrimidine-rich tract.

3. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises a sequence corresponding to a region selected from the group consisting of BoxA, BoxB, a trans factor-binding site, and a combination thereof.

4. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region further comprises an AUG or ATG sequence.

5. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein the 5'-untranslated region comprises a part of or an entire region of IRES (internal ribosomal entry site) of viral mRNA.

6. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1 further comprises a portion of a coding region adjacent to the 5'-untranslated region, or a fragment or a variant thereof, of a viral gene in addition to said nucleic acid sequence.

7. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid sequence for enhancing expression of a useful gene is incorporated downstream of an expression regulation promoter sequence and upstream of the useful gene in a gene expression vector.

8. The nucleic acid sequence for enhancing expression of a useful gene according to

claim 1, wherein said nucleic acid is a cDNA sequence.

9. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said gene expression vector is a vector for expression in eukaryotic cells.

10. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said virus is RNA virus.

11. The nucleic acid sequence for enhancing expression of a useful gene according to claim 10, wherein said virus is picornavirus.

12. The nucleic acid sequence for enhancing expression of a useful gene according to claims 10, wherein said virus is HCV (hepatitis C) virus.

13. The nucleic acid sequence for enhancing expression of a useful gene according to claim 10, wherein said virus is HCV virus, and said nucleic acid sequence for enhancing expression of a useful gene further comprises a portion of the coding region for the core protein of the HCV virus or, a variant thereof.

14. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of the following nucleotide sequence:

gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgtcgtgcag cctccaggac 120
ccccctccc gggagagcca tagtggtctg cggaaccggt ggtacaccg gaattgccag 180
(SEQ ID NO: 1, 1-180).

15. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of the following nucleotide sequence:

gacgaccggg tcctttcttg gatcaacccg ctcaatgcct ggagatttgg gcgtgcccc 60
gcgagactgc tagccgagta gtgttggggtc gcgaaaggcc ttgtggtact gcctgatagg 120
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac c 161
(SEQ ID NO: 1, 181-341).

16. The nucleic acid sequence for enhancing expression of a useful gene according to claim 12, wherein said nucleic acid sequence consists of the following nucleotide sequence:

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gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgtcgtgcag cctccaggac 120
ccccctccc gggagagcca tagtggctct cggaaccggt gagtacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaaccgc ctcaatgcct ggagatttgg gcgtgcccc 240
gcgagactgc tagccgagta gtgttgggtc gcgaaaggcc ttgtggtact gcctgatagg 300
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac c 341
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(SEQ ID NO: 1, 1-341).

17. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of the following nucleotide sequence:

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gacgaccggg tcctttcttg gatcaaccgc ctcaatgcct ggagatttgg gcgtgcccc 60
gcgagactgc tagccgagta gtgttgggtc gcgaaaggcc ttgtggtact gcctgatagg 120
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac catgagcaca aatcctaaac 180
ctcaaagaaa aaccaaactg aacaccaacc gccgccaca ggacgtcaag tccccgggcg 240
gtggtcagat cggttgggtg gtttacctgt tgccgcgcag gggccccagg ttgggtgtgc 300
gcgcgactag gaagacttcc gagcgtcgc aacctcgtgg aaggcgacaa cctatcccca 360
aggtcgcgcg gcccgagggc aggacctggg ctacgcccg gtatccttgg cccctctatg 420
gcaacgaggg catggggtgg gcaggatggc tcctgtcgcc ccgcggctcc cggcctagtt 480
ggggcccttc ggacccccgg ctaggtcgc gtaatttggg taaggtcac gat 533
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(SEQ ID NO: 1, 181-713).

18. The nucleic acid sequence for enhancing expression of a useful gene according to claim 13, wherein said nucleic acid sequence consists of the following nucleotide sequence:

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gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg 60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgtcgtgcag cctccaggac 120
ccccctccc gggagagcca tagtggctct cggaaccggt gagtacaccg gaattgccag 180
gacgaccggg tcctttcttg gatcaaccgc ctcaatgcct ggagatttgg gcgtgcccc 240
gcgagactgc tagccgagta gtgttgggtc gcgaaaggcc ttgtggtact gcctgatagg 300
gtgcttgcca gtgccccggg aggtctcgta gaccgtgcac catgagcaca aatcctaaac 360
ctcaaagaaa aaccaaactg aacaccaacc gccgccaca ggacgtcaag tccccgggcg 420
gtggtcagat cggttgggtg gtttacctgt tgccgcgcag gggccccagg ttgggtgtgc 480
gcgcgactag gaagacttcc gagcgtcgc aacctcgtgg aaggcgacaa cctatcccca 540
aggtcgcgcg gcccgagggc aggacctggg ctacgcccg gtatccttgg cccctctatg 600
gcaacgaggg catggggtgg gcaggatggc tcctgtcgcc ccgcggctcc cggcctagtt 660
ggggcccttc ggacccccgg ctaggtcgc gtaatttggg taaggtcac gat 713
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(SEQ ID NO: 1, 1-713).

19. The nucleic acid sequence for enhancing expression of a useful gene according to claim 3, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence or a proximate sequence in at least one position corresponding to a pyrimidine-rich tract, BoxA, BoxB and/or trans factor-binding site contained in the 5'-untranslated region.

20. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid comprises a sequence having substitution, deletion, insertion and/or addition of a single or a few nucleotides of a sequence derived from a wild-type virus within the sequence corresponding to a region other than the 5'-untranslated region.

21. The nucleic acid sequence for enhancing expression of a useful gene according to claim 15, wherein said nucleic acid has one thymidine inserted into position 207 of SEQ ID NO: 1.

22. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of its own translation promoting activity.

23. The nucleic acid sequence for enhancing expression of a useful gene according to claim 1, wherein said nucleic acid sequence for enhancing expression of a useful gene enhances expression of a useful gene by means of accelerating IRES activity.

24. A nucleic acid sequence for enhancing expression of a useful gene comprising the following nucleotide sequence:

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gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg    60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgctgtgcag cctccaggcc    120
ccccctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag    180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc    240
cgcgagactg ctagccgagt agtgttgggt cgcgaaaggc cttgtggtac tgctgatag    300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc                        342
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(SEQ ID NO: 7), which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

25. A nucleic acid sequence for enhancing expression of a useful gene which comprises a polynucleotide having a similar IRES activity to an IRES activity of the following nucleotide sequence:

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gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg    60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc    120
ccccctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag    180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc    240
cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgccatgatag    300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc                        342
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(SEQ ID NO: 7), and consisting of a fragment or a variant of the sequence, which enhances expression of a useful gene by means of promoting mRNA translation in an IRES-dependent manner.

26. An isolated polynucleotide consisting of the following nucleotide sequence:

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gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg    60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc    120
ccccctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag    180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc    240
cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgccatgatag    300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc                        342
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(SEQ ID NO. 7).

27. An isolated polynucleotide having a similar IRES activity to an IRES activity of the following nucleotide sequence:

```
gccagcccc tgatgggggc gacactccac catagatcac tcccctgtga ggaactactg    60
tcttcacgca gaaagcgtct agccatggcg ttagtatgag tgcgtgcag cctccaggcc    120
ccccctccc gggagagcca tagtggtctg cggaaccggt gagtacaccg gaattgccag    180
gacgaccggg tcctttcttg gatcaatccc gctcaatgcc tggagatttg ggcgtgcccc    240
cgcgagactg ctagccgagt agtggtgggt cgcgaaaggc cttgtggtac tgccatgatag    300
ggtgcttgcg agtgccccgg gaggtctcgt agaccgtgca cc                        342
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(SEQ ID NO: 7), and consisting of a fragment or a variant of said sequence.

28. A gene expression vector comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 1.

29. A host cell transformed or transfected with the vector according to claim 28.

30. A method of expressing a useful gene product using the vector according to claim 28.

31. A method for producing a useful gene product comprising the steps of: growing the host cell according to claim 29 in a medium; and isolating the useful gene product from the cell and/or the growth medium.

32. A method for enhancing expression of a useful gene product using the vector according to claim 28.

33. A probe for screening substances that interact with IRES, comprising the polynucleotide according to claim 26.

34. A probe for screening IRES-dependent translation initiators, comprising the polynucleotide according to claim 26.

35. A therapeutic composition for treating diseases resulting from reduction of cap-dependent mRNA translation in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 1 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

36. A therapeutic composition for treating diseases resulting from reduction of IRES activity in a body of organisms, comprising the nucleic acid sequence for enhancing expression of a useful gene according to claim 24 such that translation of mRNA can be promoted by means of introducing said nucleic acid sequence for enhancing expression of a useful gene into the body of the organisms.

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37. A method for determining the severity of hepatitis C, comprising the steps of:
detecting the presence of a target polynucleotide sequence contained in a biological sample
derived from a test subject, by using the polynucleotide according to claim 26 as the target; and
determining the severity of the hepatitis C based on the presence of the sequence.

38. The nucleic acid sequence for enhancing expression of a useful gene according to
claim 16, wherein said nucleic acid has one thymidine inserted into position 207 of SEQ ID NO:
1.

39. The nucleic acid sequence for enhancing expression of a useful gene according to
claim 17, wherein said nucleic acid has one thymidine inserted into position 207 of SEQ ID NO:
1.

40. The nucleic acid sequence for enhancing expression of a useful gene according to
claim 18, wherein said nucleic acid has one thymidine inserted into position 207 of SEQ ID NO:
1.

41. A probe for screening substances that interact with IRES, comprising the
polynucleotide according to claim 27.

42. A probe for screening IRES-dependent translation initiators, comprising the
polynucleotide according to claim 27.

43. A method for determining the severity of hepatitis C, comprising the steps of:
detecting the presence of a target polynucleotide sequence contained in a biological sample
derived from a test subject, by using the polynucleotide according to claim 27 as the target; and
determining the severity of the hepatitis C based on the presence of the sequence.